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Genetic analysis of a novel Alaska barley yellow dwarf virus in the family *Luteoviridae*

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Summary. A new plant virus belonging to the family *Luteoviridae* and isolated from diseased oat (*Avena sativa* L.) plants was discovered in Alaska in 2003. Even though plants with red/orange leaves were indicative of barley yellow dwarf disease, they were not reactive to specific antibodies corresponding to barley yellow dwarf virus (BYDV)-MAV, -PAV, -SGV, and cereal yellow dwarf virus-RPV from enzyme-linked immunosorbent assays (ELISA). An alternative RT-PCR assay that incorporated Shu-F/Yan-R primers for detection of BYDV-MAV, -PAS, -PAV, and SGV was effective in producing ∼830-nt fragments that contained genomic sequences to the 3′-terminus of the polymerase gene (ORF 2), the intergenic region (∼113 nt), the coat protein gene (ORF 3), and the putative movement gene (ORF 4). The Alaskan isolates were most similar to BYDV-MAV with only about 77 and 80% amino acid identity in the CP and ORF 4, respectively. The Alaska isolates coat protein gene sequences differed in several regions that otherwise are conserved among BYDV-MAV isolates, and may be important in serological variations, accounting for the negative ELISA results. Based upon sequence and serological differences, we concluded that the Alaskan BYDV-MAV-like isolates formed a novel species tentatively in the genus *Luteovirus*, and propose the name BYDV-ORV (oat red-leaf virus).

Introduction

Barley yellow dwarf (BYD) disease exclusively afflicts plant species in the grass family, *Poaceae*, and is especially noticeable wherever crops such as barley, maize,

Nucleotide sequence data reported are available in the DDBJ/EMBL/GenBank databases under the accessions numbers DQ680114–DQ680140, DQ683252, DQ792506.

oats, rye, rice, and wheat are cultivated [3]. At least eight viruses belonging to the family *Luteoviridae* cause BYD, and are directly responsible as separate or multiple entities for economic losses incurred in the cereals and important range grasses [16]. Diseased plants may exhibit prominent yellow/red discolorations on leaves, extreme plant stunting, and reduced yield in grain. Most species were named, in part, after their most efficient aphid vector and taxonomically classified as: 1) genus *Luteovirus*: BYDV-MAV (*Sitobion* (*Macrosiphum*) *avenae*), BYDV-PAS (formerly PAV-129; *Rhopalosiphum padi*), BYDV-PAV (*R. padi & S. avenae*), and BYDV-RGV (rice giallume), 2) genus *Polerovirus*: *Cereal yellow dwarf virus*-RPV (formerly BYDV-RPV; *R. padi*), and 3) unassigned genera: BYDV-GPV (*Schizaphis graminum* & *R. padi*), BYDV-RMV (*R. maidis*), and BYDV-SGV (*S. graminum*) [4, 19]. The species are distinguished by gene arrangement and sizes on a single-stranded RNA genome with 5.3–5.8 kb [4, 19]. The 5′-half consists of two major overlapping genes involved in replication (ORF 1 and 2), is conserved within a genus, and shares features of the polymerase gene with either carmoviruses or sobemoviruses; CYDV has an extra ORF (0) at the 5′ end. In contrast, the 3′-half is the most conserved portion of the genome among genera, containing the "Luteoviridae block" that consists of ORF 4 situated within the major coat protein gene (ORF 3) that is fused to ORF 5, resulting in a read-through domain; BYDVs have an extra ORF (6) at the 3′ terminus [22].

Detection and identification of B/CYDVs have been based on serology (ELISA, enzyme-linked immunosorbent assay), aphid transmission studies, and, more recently, PCR (polymerase chain reaction) assays with genomic sequences [8]. Field surveys involving B/CYDVs in epidemiological studies, aphid vector assessments, and specific species identification usually employ ELISA to process large number of samples quickly and fairly accurately [6, 7]. Conflicting results and confusion may occur in the detection of variants and members of previously unidentified species such as BYDV-PAS [2, 22]. Also, different "lots" of antiserum may vary in sensitivity and consistency. A relatively large number of samples can be processed using PCR assays that are more sensitive than serological assays, with little concern for "lot" differences, and provide fragment copies of the genome for definitive sequence determination. Over a decade and a half ago, a universal luteovirus primer set, Lu1/Lu4, that spanned most of the coat protein (CP) gene, was successfully employed for detection of six members of the family *Luteoviridae*: BYDV-MAV, BYDV-PAV, BYDV-SGV, CYDV-RPV, PLRV (potato leafroll virus), and BWYV (beet western yellows virus) [25]. Recent field surveys of barley and oats in Alaska have confirmed the presence of BYDV-PAV and CYDV-RPV isolates using ELISA and RT-PCR [24]. Upon sequencing the PCR products, we determined that all the isolates from south central Alaska previously identified as BYDV-PAV by serology were in fact BYDV-PAS (unpublished, N. L. Robertson & R. French). Incorporating RT-PCR assays with sequence documentation from our field surveys was essential for definitive identifications, and usually eliminated any conflicting ELISA results. However, in 2003, a field containing oat plants with classical BYD symptoms, and within several kilometers of other fields with B/CYDV-infected oat and barley

plants, proved to be problematic and quite puzzling. Initial screening of fresh plant tissue by ELISA and RT-PCR for luteoviruses detection failed in all but one sample where sequence analysis of the RT-PCR product (CP gene, accession no. DQ683252) showed that it was BYDV-PAS.

The following year, Malmstrom and Shu [17] reported a multiplexed RT-PCR protocol for detection and separation of B/CYDVs using a number of newly designed primers. A particular primer set, Yan-R/Shu-F, proved to be quite effective in detecting a virus from the described oat samples that previously had escaped from PCR and serological detections. This paper describes the detection and identification of a novel member of the family *Luteoviridae* and its occurrence in south central Alaska. The molecular diversity of the unique isolates was examined and compared with other B/CYDV isolates occurring in Alaska and throughout the world.

Materials and methods

Plant materials

During an August 2003 disease survey in the Matanuska Valley near Palmer, Alaska, an estimated one percent of the plants in a field of oats (field (site) 5) at the head-stage contained symptoms similar to barley yellow dwarf disease. Affected plants usually had bright red/orange discoloration on the younger leaves including the flag leaf, while the older leaves were green. Two/three discolored leaves were detached from each of the randomly chosen 39 plants (plus green leaves from two healthy plants), placed into labeled plastic bags, and stored at 4 °C for processing. Leaves from individual plants were cut into small pieces, and aliquots of 0.1–0.25 and 0.5 g were packaged and frozen at −80 °C.

ELISA

Samples from each plant were screened for BYDV-MAV, BYDV-PAV, and BYDV-SGV utilizing DAS (double antibody sandwich)-ELISA and compound direct for CYDV-RPV with alkaline phosphatase enzyme conjugate from Agdia PathoScreen kits (Elkhart, IN) as directed from manufacturer. Briefly, 0.1–0.25 g leaf tissue was ground in 1.0 ml distilled water with mortar and pestle, 10 µl of the extract mixed with 90 µl extraction buffer, applied to provided microtitre plates, and completed with positive and negative controls according to manufacturer directions. Further confirmation for BYDV-MAV was later tested from the same frozen leaf extracts using a DAS-ELISA kit from BIOREBA AG (Reinach, Switzerland) as instructed by the manufacturer. All assays were analyzed on a VERSAmax microplate reader (Molecular Devices, Sunnydale, CA, USA) at 405 nm with positive readings at least two times the negative control.

Polymerase chain reaction, cloning, sequencing

Total nucleic acid extracts from leaves of each of the 41 plants were processed as previously described [24] and stored at −80 °C. Two to five micro liter nucleic acid and reverse primers, Lu 4 [25] or Yan-R [17] were used in the cDNA synthesis protocol as described by Robertson et al. [25]. The following PCR assays were performed using the same plant extracts with the following primer sets: 1) Lu 1/Lu 4, targeting ∼530 nt on the CP gene (ORF 3) for all B/CYDVs, 2) Pol1/Lu 4, targeting ∼1400 nt on the polymerase (ORF 2) and CP genes from only B/CYDVs that contain sobemovirus-like polymerases such as CYDV-RPV and BYDV-RMV [11] and,

Table 1. Alaska barley yellow dwarf virus isolates detected from plants in 2003 from a field of oats by RT-PCR assays

Plant no.[a]	RT-PCR Lu1/Lu4[b]	RT-PCR Shu-F/Yan-R[c]	RT-PCR MAV2-F/Yan-R[d]	Isolate[e]	Accession number[f]
1	−	+	−		
2	−	±	±	MAV02P5o03	DQ680115
3	−	±	±	MAV03P5o03	DQ680116
4	−	±	±	MAV04P5o03	DQ680131
5	−	−	n/a		
6	−	−	n/a		
7	−	±	±	MAV07P5o03	DQ680114
8	−	±	−	MAV08P5o03	DQ680117
9	−	±	n/a		
10	−	−	n/a		
11	n/a	n/a	n/a		
12	−	±	−	MAV12P5o03	DQ680118
13	−	±	−	MAV13P5o03	DQ680119
14	−	±	±	MAV14P5o03	DQ680120
15	−	±	±	MAV15P5o03	DQ680121
16	−	±	±	MAV16P5o03	DQ680122
17	−	±	±	MAV17P5o03	DQ680132
18	−	−	n/a		
19	−	±	−	MAV19P5o03	DQ680123
20	−	+	−		
21	−	±	±	MAV21P5o03	DQ680133
22	−	±	±	MAV22P5o03	DQ680134
23	−	±	+	MAV23P5o03	DQ680124
24	−	±	+	MAV24P5o03	DQ680135
25	−	+	−		
26	−	±	±	MAV26P5o03	DQ680138
27	−	±	±	MAV27P5o03	DQ680126
28	−	±	−	MAV28P5o03	DQ680127
29	−	±	−	MAV29P5o03	DQ680128
30	−	±	±	MAV30P5o03	DQ680129
31	−	−	n/a		
32	−	−	n/a		
33	−	−	n/a		
34	−	−	n/a		
35	−	−	n/a		
36	−	−	n/a		
37	±		−	PAS37P5o03_clone 1 PAS37P5o03_clone 2	DQ683252 DQ792506
38	−	±	±	MAV38P5o03	DQ680136
39	−	±	±	MAV39P5o03	DQ680130
40	−	−	n/a		
41	−	−	n/a		
Total	1/41	27/41	16/27	23 BYDV-MAV-like 1 BYDV-PAS-like	

[a]Plant sample number corresponding to isolate number; no. 11, lost nucleic acid extract
+ = obtained predicted size of fragment; ± = obtained sequences from fragment

[b]PCR product ~530 bp

[c]PCR product ~830 bp

[d]PCR product ~590 bp

[e]MAV or PAS = similar to BYDV-MAV or -PAS, XX (isolate number), P (Palmer (Alaska) = location), 5 (field number), o (oat = natural plant host), 03 (year collected)

[f]Nucleotide sequence obtained from central portion of PCR 830 fragment on both strands varying from 575 to 783 nts, except accession number DQ683252 (isolate PAS37P5o03_clone 1) is from central portion of PCR 530 fragment

3) Shu-F/Yan-R, targeting ∼830 nt on the polymerase and the cp genes in BYDV-MAV, BYDV-PAV, or BYDV-SGV; PCR products of ∼830 bp were further analyzed for specific BYDV-MAV, BYDV-PAV, and BYDV-SGV identification with MAV2-F/Yan-R, PAV-F/Yan-R, and Shu-F/SGV-R primers, respectively [17]. Modifications to the standard PCR protocol [25] for the reactions with species-specific primers included an increased amount of primers to 20–100 pmol, and an elevated annealing temperature (55 °C). Samples were placed in Gen Amp® PCR System 9700 (PE Applied Biosystems, Foster City, CA). The amplified DNA fragments were purified with QIAquick PCR Purification kit (QIAGEN Inc., Valencia, CA, USA), and visualized on ethidium-bromide-stained agarose gels for size and concentration determinations. The purified DNA samples and Shu-F, Yan-R, and MAV-F primers were submitted to Davis Sequencing, Inc. (Davis, CA) for direct sequencing of both DNA strands. The PCR fragment of ∼530 bp derived from plant no. 37 with Lu1/Lu4 primers was cloned into pGem-T Easy (Promega, Madison, WI), transformed into *Escherichia coli* XL-2 Blue (Stratagene, La Jolla, CA), and sequenced on both strands. We were curious if BYDV-MAV-like isolates were present on other sites and, in addition to oats, if barley was also a host. Barley and oat plants from two different sites in 2003 and 2004 (within several kilometers of field 5) were specifically assayed for MAV detection using stored (−80 °C) nucleic acid extracts, MAV2-F/Yan-R primers and processed as previously described.

Nucleotide sequences were obtained on both strands from PCR-generated Shu-F/Yan-R fragments for 24 isolates: sequences were also obtained on one strand from MAV2-F/Yan-R-generated fragments for 14 of the 16 additional isolates in Table 1. All sequences were first examined and compared with each other using the Sequencher program (Gene Codes Corp., Ann Arbor, MI, USA), followed by a quick identity assessment with other viruses using BLAST (basic local alignment search tool), nucleotide-nucleotide (blastn) program in NCBI (National Center for Biotechnology Information).

Genetic analysis

Sequence alignments were completed by the Clustal_X program [27]. Neighbor-joining trees for the CP (Fig. 2a) and ORF 4 (Fig. 2b) were based on distances of net amino acid sequence differences and constructed with PAUP (Phylogenetic Analysis Using Parsimony) [26]. Distribution of amino acid sequence similarities on the CP among selected BYDV isolates that represented each clade from the CP phylogenetic tree (Fig. 2a) were visualized with a Hypercard computer program [9] using a sliding window of 10 amino acids (Fig. 3). Identities for CP nucleotide sequences and amino acid sequences for the CP and ORF 4

Table 2. Percent sequence identities within (on the diagonal in bold) and between (below the diagonal) BYDV phylogenetic clusters, exclusive of PAV CN and Saldus1. The first number is percent nucleotide identity of the coat protein gene, and the second and third numbers are percent amino acid identities of the coat protein and ORF 4, respectively. As only one SGV sequence was used as the outgroup, within group diversity values are not applicable (na) for SGV

Viruses* no. of isolates	AK MAV-like			MAV			PAV			PAS			SGV		
AK MAV-like* (27)	**98.3**	**97.2**	**99.4**												
MAV* (6)	83.2	77.3	80.2	**98.5**	**97.9**	**98.3**									
PAV* (7)	74.4	64.1	70.1	77.1	72.4	70.5	**95.8**	**96.3**	**90.4**						
PAS* (4)	74.2	64.9	73.5	77.3	71.1	73.0	88.3	84.8	87.7	**96.8**	**92.9**	**97.6**			
SGV* (1)	69.0	57.9	64.4	67.7	58.8	59.0	67.1	59.9	59.7	67.9	57.8	57.5	na	na	na

(Table 2) were determined among 27 Alaskan isolates collected in 2003 (23) and 2004 (4), and BYDV isolates from other geographical regions using the MEGA3.1 computer program [14].

Results

ELISA

None of the leaf sap preparations derived from 41 oat plants reacted to the specific antibodies from BYDV-MAV, BYDV-PAV, BYDV-SGV, or CYDV-RPV in ELISA tests. Extracts from all plants and controls were assayed at least three times for confirmation.

Sequences of PCR products

The "universal luteovirus" and "sobemovirus-like polymerase" primer sets failed to generate amplified DNA fragments from any of the 41 oat plants from field 5, with one exception, using the former primer set (Fig. 1, Table 1). However, about two years later, successful RT-PCR detection of BYD using the same nucleic acid extracts with Yan-R primer for cDNA synthesis, and Shu-F/Yan-R primers in PCR, resulted in amplified DNA fragments of the predicted size ~830 bp (Fig. 1, Table 1). In fact, nearly 68% (27 out of 41) of the collected plants were tentatively identified to have been infected with at least one of the following: BYDV-MAV, BYDV-PAS, BYDV-PAV, or BYDV-SGV; sequences were obtained from 24 isolates as indicated with corresponding accession numbers. Subsequently, sixteen of these isolates were tentatively identified as BYDV-MAV-like from

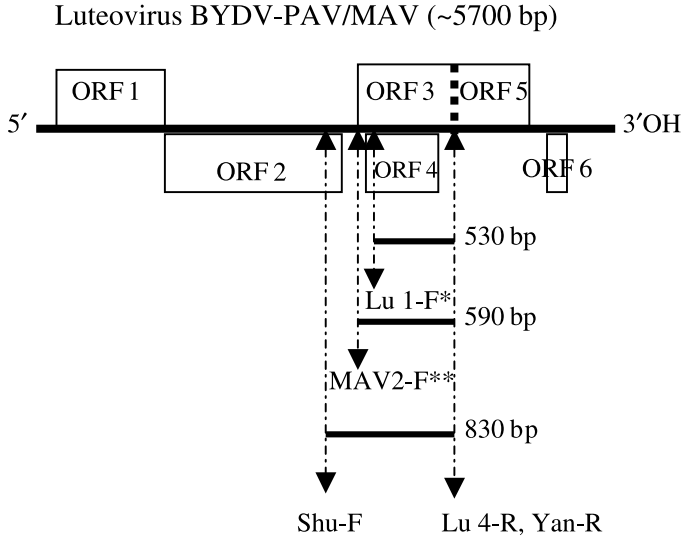


Fig. 1. Genomic map of members of the genus *Luteovirus*, depicting sequence placement of primers that successfully generated PCR fragments of predicted sizes; sequence mismatch and failure to produce PCR fragments for *MAV-like or **PAV-like genomes

the detection of 590-bp fragments generated with MAV2-F/Yan-R by PCR, and 14 of these were successfully sequenced; no PCR fragments occurred from the other isolates with MAV2-F/Yan-R, PAV-F/Yan-R, or SGV-R/Shu-F primers sets. The other sites within several kilometers of the original "MAV-site" that were

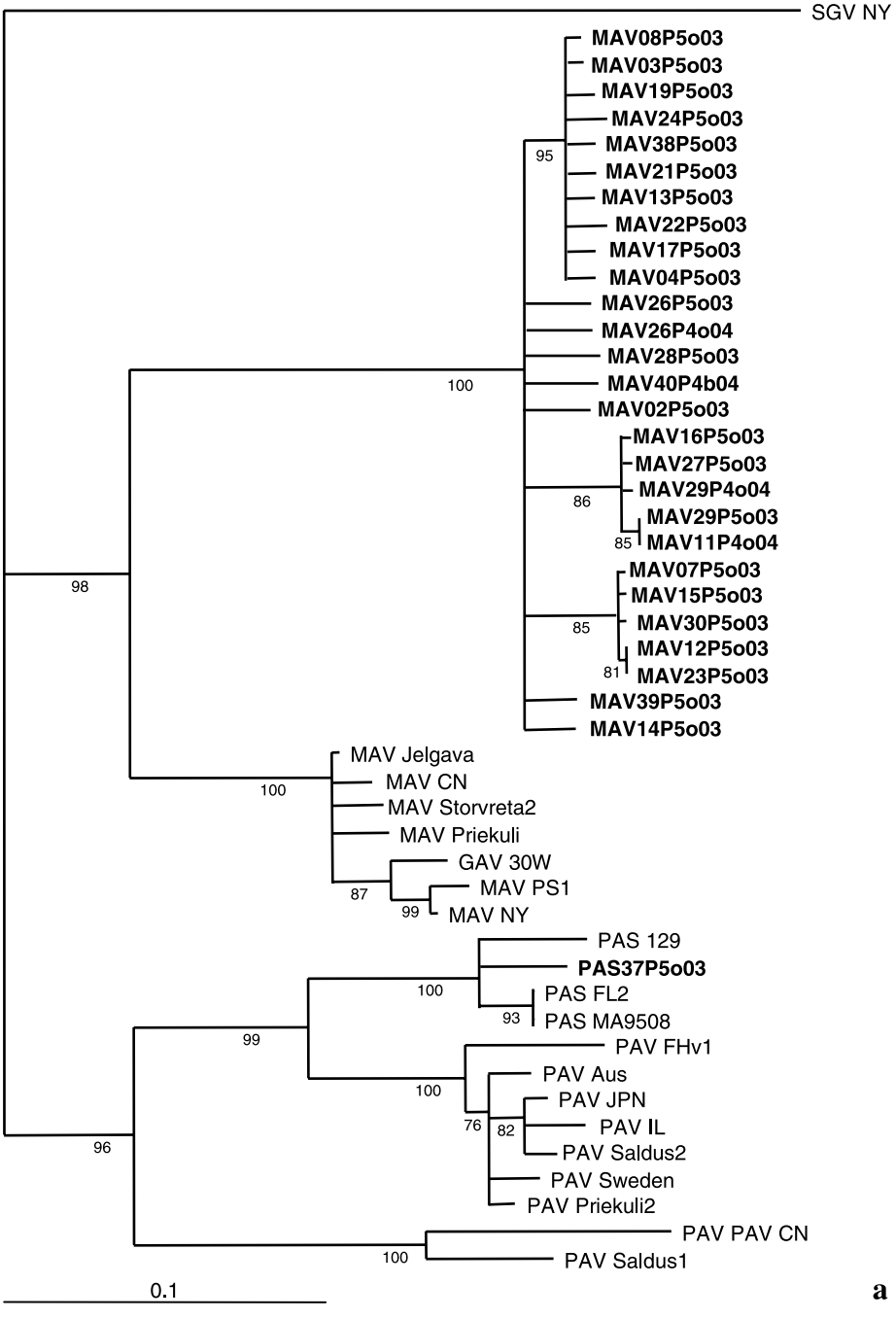
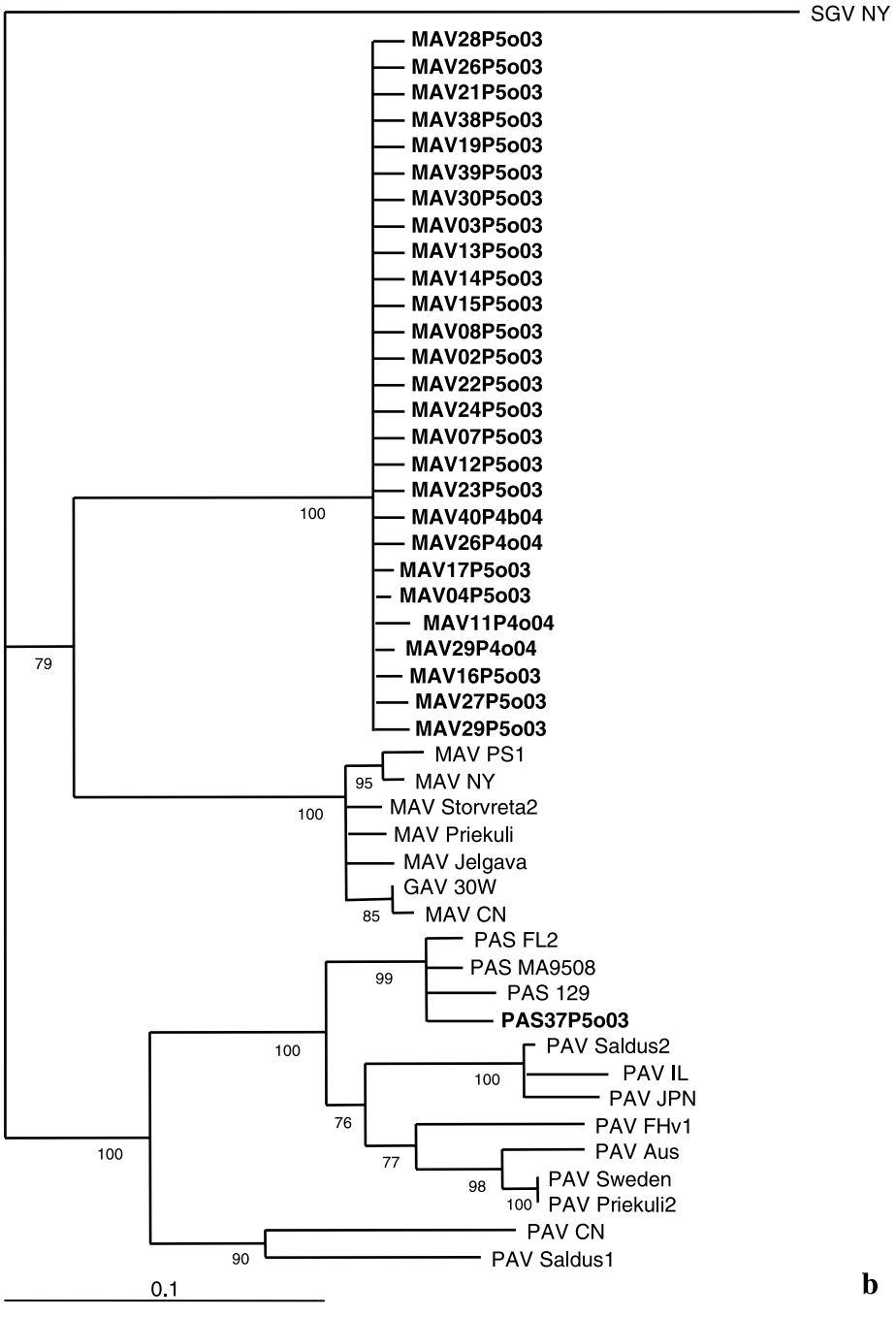


Fig. 2 (*continued*)

assayed in 2003–04 only contained MAV-like isolates from 2004, including one isolate (MAV40P4b04, accession no. DQ680140) from 13 barley plants and three isolates (MAV11P4o04, accession no. DQ680137; MAV26P4o04, accession no. DQ680138; MAV29P4o04, accession no. DQ680139) from 33 oat plants. The four 2004 isolates contained sequences similar to each other and to the AK BYDV-MAV-like isolates from 2003 (Fig. 2a, b, Table 2).



All AK isolates except one (PAS37P5o03) aligned together with Sequencher with limited mismatches and were most similar to BYDV-MAVs sequences when analyzed with blastn. Two clones (accession nos. DQ683252, DQ792506) derived from plant no. 37 (isolate PAS37P5o03) using Lu 1/Lu 4 and Shu-F/Yan-R primers, respectively, contained nearly identical 502-nt (one mismatch) between the Lu1 and Lu 4 sites. The larger PAS37P5o03 clone (DQ792506) contained nucleotide sequences (49–163, 164–757, 206–667 nt) that were 96, 98, and 98% identical to the intergenic region, ORF 3 (lacking 3′-terminus of CP gene), and ORF 4, respectively, with the New York BYDV-PAS (accession no. AF218798, 2744–2857, 2858–3452, 2901–3362 nt, respectively).

Phylogenetically, all 27 AK BYDV-MAV-like isolates clustered in one clade when analyzing amino acid sequences among other BYDV species in the CP (Fig. 2a) and ORF 4 (Fig. 2b). These isolates were 98.3 and 97.2% identical in nucleotide (nt) and amino acid (aa) sequences of the CP gene, respectively, and more conserved in the ORF 4 protein with a 99.4% identity (Table 2). When compared to other BYDV isolates, the AK isolates were most similar to BYDV-MAV, with sequence percent identities of 83.2, 77.3, and 80.2 in the CP nt, CP aa, and ORF 4 aa, respectively (Table 2). The putative movement protein, ORF 4, was more conserved relative to the CP, with a difference of +2.2, +0.4, and +4.7% in the AK MAV-like, MAV, and PAS clade, and less conserved in the PAV clade at −5.9% (Table 2).

The distribution of amino acid differences along the CP was obvious when comparing sequences of an AK MAV-like isolate (MAV07P5o03) with representative members from the distinct clades (Fig. 3). Notably, differences between the three AK isolates and the other BYDV isolates occur on the 5′-termini. The AK isolates are most similar to MAV PSI and GAV 30W near the 3′-termini.

The AK BYDV-MAV-like isolates contained a 113-nt intergenic region between the polymerase (ORF 2) and CP (ORF 3) genes that were at least 95% similar

Fig. 2. Phylogenetic trees depicting relationships among 27 *Barley yellow dwarf virus* (BYDV)-MAV-like isolates (accession numbers: DQ680114–DQ680140) and one PAS-like isolate (accession no. DQ792506) from Alaska and other selected BYDV-MAV, -GAV, -PAS, -PAV isolates comparing amino acid sequences of the CP gene, ORF 3 (Fig. 2a), or the putative movement gene, ORF 4 (Fig. 2b). The Alaska isolates are in bold as: **MAV** or **PAS**, **xx** (sample number), **P** (Palmer = location), **o** or **b** (oat or barley = plant host), **03** or **04** (year collected). The associated country and accession number are included with the other isolates: GAV 30w (China, AY610953), MAV CN (China, AF338909), MAV Jelgava (Latvia, AJ563519), MAV NY (USA, X53174), MAV PSl (USA, D11028), MAV Priekuli (Latvia, AJ563417), MAV Storvreta2 (Sweden, AJ563417), PAS 129 (USA, AF218798), PAS FL2 (France, AJ223586), PAS MA9508 (Morocco, AJ007921), PAV Aus (Australia, M21347), PAV CN (China, AF192967), PAV FHv1 (France, AJ007491), PAV IL (USA, AF235167), PAV JPN (Japan, D85783), PAV Priekuli2 (Latvia, AJ563414), PAV Saldus1 (Latvia, AJ563410), PAV Saldus2 (Latvia, AJ563411), PAV Sweden (Sweden, AJ563415) and SGV NY (USA, U06865). The scale bar (0.1) means a distance of 10% with both trees drawn to the same scale. Bootstrap values are percent results from 1000 bootstrap replications

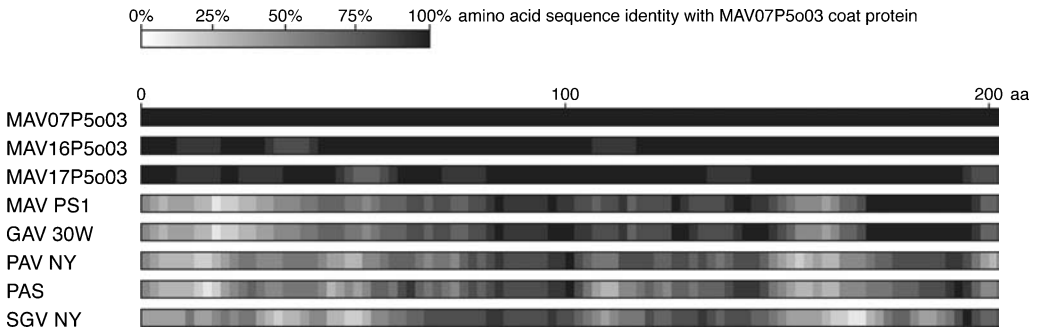


Fig. 3. Coat protein amino acid sequence comparisons and distributions of an Alaska BYDV-MAV-like isolate (MAV07P5o03, accession no. DQ680114) with two AK MAV-like isolates (MAV16P5o03, DQ680122; MAV17P5o03, DQ680132), and five other BYDV isolates (accessions nos. in parenthesis): MAV PSI (X17260), GAV30W (AY610953), PAV NY (X56050), PAS (AF218798) and SGV NY (U06865)

to each other, and, interestingly, more similar to BYDV-PAS-129 (85.9–87.0%) and BYDV-PAV NY (82.8–83.8%) than to BYDV-MAV-PSI (81.3–82.3%).

Discussion

Failure to initially detect B/CYDVs from field plants with classical BYD symptoms by two very specific and sensitive detection methods presented a dilemma that is occasionally encountered in field diagnostics. We had tentatively concluded that the sampled oats plants did not have BYD disease, but rather were infected by another pathogen such as aster yellows phytoplasma, or had a nutrient deficiency that caused similar symptoms [3]. Similar detection problems involving large numbers of symptomatic plants did not occur on other sites in the Matanuska Valley, where infections by BYDV-PAV and CYDV-RPV were readily confirmed by ELISA and RT-PCR [23]. Bisnieks [1] reported successful detection and amplification of the CP gene for BYDV-PAV and CYDV-RPV, and not isolates of BYDV-MAV, using immunocapture RT-PCR with the appropriate antiserum and the universal luteovirus primers. They subsequently incorporated a specific primer set for successful BYDV-MAV amplification and analysis. As previously stated, Malmstrom and Shu [17] developed a multiplexed RT-PCR strategy for detection and separation of B/CYDV serotypes using multiple primers sets. They concluded that their MAV2-F (5′-AATAACCGCCAGGAGAAATGG-3′) and Yan-R primer pair were specific for BYDV-MAV and not BYDV-PAV and BYDV-SGV. Upon examination of the nucleotide sequences in the AK BYDV-MAV-like isolates and available sequences for BYDV-MAV from data banks, the suitability and application of the various primers designed by different researchers for detection became evident. Firstly, the universal Lu 1 primer, 5′-CCAGTGGTTRT GGTC-3′, was not useable for detection for any BYDV-MAV [1, 15, 17], including the AK BYDV-MAV-like isolates, since there was no sequence counterpart in the coat protein region, with a specific mismatch in the equivalent region,

5′-**A**CAGTGGTTATGG**CA**-3′. Interestingly, if BYDV-MAV sequence information had been available for the original design of the universal luteovirus primers [25], Lu 1 probably would not have been formulated. Ironically, the Lu 1/Lu 4 primers continue to be exceedingly useful and dependable for sensitive detection of most members of the family *Luteoviridae*, and for finding new viruses such as sweet potato leaf speckling virus [10], and more recently, BYDV-OYV (oat yellowing virus) = PAV-Sal1 [1].

Secondly, the primer set designed by Bisnieks [1] that specifically targets BYDV-MAVs would not be useful in detecting the Alaska isolates since the M3 forward primer, 5′-ATG AAT TCA GTA GGC C**G**T A**G**-3′, contained two nucleotide mismatches (underlined) at critical locations on the 3′-terminus for proper binding to Alaska MAV-like isolates, 5′-ATG AAT TCA GTA GGC C**T**T A**A**-3′ (i.e. MAV07P5o03, accession no. DQ680114, 194–213 nt). Finally, the primer set instrumental in diagnosing the problematic oat plants in Alaska, Shu-F/Yan-R (Table 1) was designed to detect BYDVs that share features with carmoviruses in their RNA-dependent RNA polymerase (ORF 2) such as BYDV-MAV, -PAV, and -SGV. The sequences obtained from the resulting PCR fragments were obviously most similar to BYDV-MAV and distantly related to BYDV-PAV. This is in contrast to the negative results with the primers, Pol 1/Lu 4, which targeted BYDV-causing viruses with a sobemovirus-like polymerase such as BYDV-RMV and BYDV-RPV. Partial success with the MAV specific primer, MAV2-F (5′-AAT AAC CGC AGG AGA AAT GG-3′), provided the correctly sized amplified fragment on 16 of the 27 isolates previously amplified with Shu-F/Yan-R primers and suggested that 16 plants were in fact infected with BYDV-MAV. However, since the Alaska isolates had little sequence similarity with the forward primer's 5′-half (underlined, bold), 5′-**AGA AAG AGG AA**G AGA AAT GG-3′ (i.e. MAV07P5o03, accession no. DQ680114, 221–237 nt), the species identity as BYDV-MAV was questionable. Conclusions as to species identity based on size of PCR products without sequence analysis need to be scrutinized, especially in surveys involving a large number of isolates where it may not be practical to obtain sequences for all "positive" fragments.

Essentially, amino acid regions along the CP of B/CYDVs determine serological affinities with defining epitopes on the virion surface [21, 23]. Unlike most plant viruses, the coat protein gene is the most conserved among the luteoviruses and explains frequent cross reactions between them [5]. The antisera used in this study specifically distinguished BYDV-MAV, -PAV, and CYDV-RPV using polyclonal antibodies for capture and monoclonal antibodies for detection, avoiding cross reactivity. Obviously, the Alaska BYDV-MAV-like isolates did not share epitopes responsible for binding to the monoclonal antibodies that were utilized in the ELISA. Even though Alaska isolates appeared to be most similar to BYDV-MAVs and BYDV-GAV, pronounced differences in amino acid segments in the CP, especially in the amino terminus (Fig. 3) most likely accounted for the lack of serological detection.

Based on prior field surveys in Alaska, incidence of BYDV-PAS-infected plants was overwhelmingly most prevalent in both oats and barley (unpublished,

N. L. Robertson and R. French). It is curious that out of 41 plants from the oat field, only one plant was infected with BYDV-PAS, 26 plants with BYDV-MAV-like isolates, and no plants contained multiple infections. Why a susceptible plant species is infected by a particular C/BYDV is complex, involving intimate relationships between the plant host, specific aphid vector, infecting virus, and influential environmental parameters. The phenomenon of cross protection in plants have been documented for BYD, whereby multiple infections may occur with unrelated viruses such as BYDV-PAV and CYDV-RPV, or are greatly reduced with closely related viruses such as BYDV-MAV and BYDV-PAV [29]. Multiple infections of viruses have never been documented among viruses with similar polymerase genes [20], and the correlation with cross protection, or controlling mechanism(s) are not understood [12]. If, in fact, AK BYDV-MAV-like isolates were closely related to Alaska BYDV-PAS, then, most likely, cross protection would have occurred between the two viruses, and the plant likely was infected with the first delivered virus. Other factors that may influence the incidence of infection, but are not known for Alaska's B/CYDVs, are the similarities and differences of native aphid vectors and alternative plant hosts.

The phylogenetic relationship depicted between the AK BYDV-MAV-like isolates and BYDV-MAV, -PAS, and -PAV isolates is suggestive that the AK isolates should be placed in the genus *Luteovirus* (Fig. 2a, b). The AK BYDV-MAV-like isolates also have a short intergenic region between the polymerase and cp genes (113 nt) that is comparable with the luteovirus members, and unlike the longer intergenic region ($\sim$200 nt) common to members of the genus *Polerovirus* [4, 20]. Another unassigned member, BYDV-GAV, is prevalent in China and serologically related to BYDV-MAV and contains similar sequences in the ORFs, except for ORF 5 and 6 [13, 28]. Based on the sequence information available for the Alaska BYDV-MAV-like isolates, we concluded that the similarly related BYDV-GAV and BYDV-MAV have the greatest affinity to the AK isolates when compared to other viruses in the data banks. However, inclusion of these AK isolates with a particular luteovirus species including BYDV-GAV and BYDV-MAV is not allowed since they share less than 90% amino acid identity in the CP and ORF 4 and therefore do not meet the criteria for placement into an existing species [4]. The full-length genomic sequence of the Alaska BYDV-MAV-like isolates will provide additional sequence data for other important genes such as the polymerase gene and the overall genomic organization required for their classification and taxonomic placement with other viruses [22]. It could also lead into speculative ancestral recombination events such as between PAS and MAV in the generation of the novel AK BYDV-MAV-like isolates. We propose to name the described Alaskan BYDV-MAV-like isolates after their apparent host preference and associated red colour, BYDV-ORV (ORV for oat red-leaf virus).

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